The foregoing notes, incomplete as they are (being based on only about eleven hours of travel), may be useful to those who may hereafter study Louisiana vegetation more intensively; and they illustrate a method of making observations in comfort in an interesting area where mosquitoes and scarcity of water might make traveling on foot rather disagreeable in summer.

THE VALUE OF NUTRIENT SOLUTIONS AS CULTURE MEDIA FOR FERN PROTHALLIA*

By Elizabeth Dorothy Wuist Brown

The value of nutrient solutions as culture media for growing fern prothallia under experimental conditions being so well known, it is the purpose of this paper to emphasize the value of these solutions for growing prothallia for class use. Excellent cultures may be obtained by using soil, peat and various other media, but it has been the writer's experience that the work is greatly simplified by the use of the nutrient solution. For after the solutions have been prepared and the cultures set up under the best light conditions available, little attention need be paid to them.

Aside from the time-saving element in caring for the cultures is the advantage of having an abundance of material in various stages of development always at hand. In this way it is possible for the student to follow the development of the prothallia from the one-cell stage to the adult form bearing antheridia, archegonia and sporophytes. This may be accomplished by varying the time of sowing the spores in the different cultures. It is well to learn the length of time required for the germination of the spores and the development of the prothallia of the particular species used before setting up the cultures for class use. The time of germination varies somewhat in different species, being more rapid in the spores containing chlorophyll.

The following solutions, Beijerinck's, Borner and Lucanus's, Knop's, Prantl's and Sachs's, proved favorable for the germina-

* Contribution from the Osborn Botanical Laboratory.

tion of the spores and the development of the prothallia of the various species of the Polypodiaceae used. However, Knop's and Prantl's solutions were on the whole the best suited, espe-

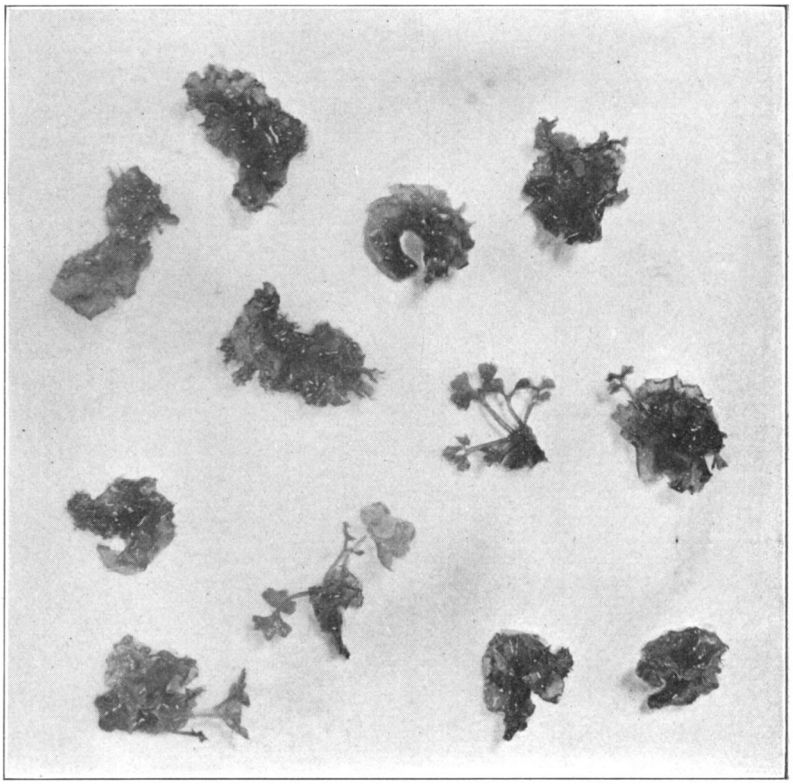

FIG. 1. Prothallia and young sporophytes of *Onoclea struthiopteris* from various nutrient solution cultures.

cially the latter as it did not seem favorable for the development of algae. The formulas for making up these solutions are as follows:

1. BEIJERINCK'S SOLUTION.

NH_4NO_3	0.5 g.
KH_2PO_4	0.2 g.
$MgSO_4$	0.2 g.
$CaCl_2$	0.1 g.
$FeCl_3$	trace.
Distilled water	1000 c.c.

2. Birner and Lucanus's Solution.

$MgSO_4$	0.5 g.
$Ca(NO_3)_2$	1.5 g.
KH_2PO_4	1.0 g.
$FeCl_3$	trace.
Distilled water	1000 c.c.

3. Knop's Solution.

$MgSO_4$	0.25 g.
$Ca(NO_3)_2$	1.00 g.
K_2HPO_4	0.25 g.
KCl	0.12 g.
$FeCl_3$	trace.
Distilled water	1000 c.c.

4. Prantl's Solution.

K_2SO_4	0.7 g.
$NaCl$	0.23 g.
$CaSO_4$	0.7 g.
$MgSO_4$	0.5 g.
NH_4NO_3 solution, 0.064 per cent	20 c.c.

5. Sachs's Solution.

KNO_3	1 g.
$NaCl$	0.5 g.
$CaSO_4$	0.5 g.
$MgSO_4$	0.5 g.
$CaHNO_4$	0.5 g.
Distilled water	1000 c.c.

Experience has shown that it is best to omit the ferric chloride from the stock solutions and to add a drop of a 1 per cent solution of ferric chloride to the nutrient solution of each culture before the spores are sown.

It is best to make up a liter of the nutrient solution, being very careful always to use only pure chemicals and distilled water. It is not necessary to sterilize the solutions, in fact cultures seem to do better on unsterilized solutions, especially those containing ammonium nitrate, probably because of chemical changes caused by heating.

Solutions should be kept in flasks or bottles well-stoppered with cotton in a clean place. Great care must be exercised in opening the flasks in the laboratory or the solutions will become

contaminated with algae or fungi. This is especially true when replenishing the culture media after the prothallia have begun to develop. Solutions should never be poured directly from the stock flasks or bottles into the culture dishes but they should be poured into a clean graduate, beaker or other receptacle and from this into the culture. In this way it is often possible to keep

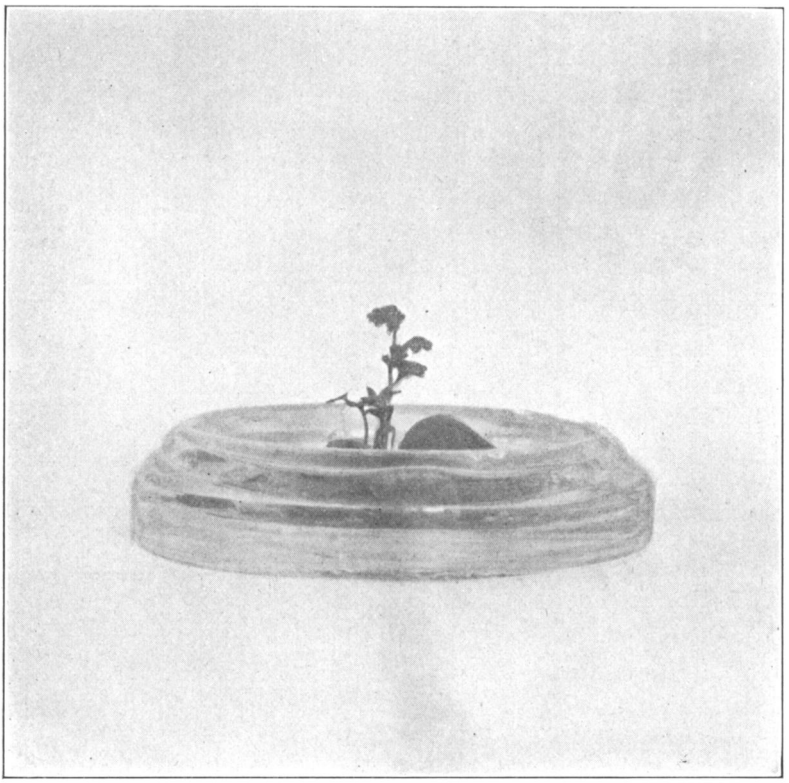

FIG. 2. Young sporophyte growing in Knop's Solution

the stock solutions pure, althoug the cultures have become contaminated. However, if they do become infested it is best to throw them away and, after cleaning and sterilizing the flasks, prepare new solutions.

In making up solution cultures it is possible to use a glass dish of any size which can be covered with a glass lid or plate, but on

the whole small glass capsules about 1¼ inches high, with a diameter of 2½ inches, seem best suited. These hold about 26 c.c. of nutrient solution. This amount of nutrient solution will provide sufficient nourishment for a large number of prothallia to grow to maturity. However, owing to evaporation, it will be necessary to add fresh solution from time to time. The advantages of using a dish of this size are many: it is easily handled by both student and teacher; it can be placed under a compound microscope and the growth of the culture observed; it does not occupy so much space on the laboratory table and therefore is not so liable to accident; and, if by chance it does meet with one or becomes too badly infected by algae or fungi, its loss is not so great. Sometimes a culture may be freed from an algal or fungous growth by lifting the prothallia with a sterilized seeker or sharp-pointed scalpel from the old solution to a new one in a clean dish. Care must be taken not to immerse the prothallia in the solution, for if this accidentally happens it will require care to make them remain on the surface afterward. Sometimes this can be accomplished by drying the upper surfaces of the prothallia with filter paper. For study by a class in beginning botany it is perhaps best to select a fern whose prothallia under normal conditions are monoecious. For this reason various species of *Aspidium* or *Camptosorus rhizophyllus* may be used, although the rarity of the latter often makes this impracticable. Among a large number of the so-called "dioecious" prothallia, especially in the older cultures, a large percentage of monoecious prothallia occur. It is also possible by allowing cultures of various species in which large, vigorous, dioecious female prothallia predominate to become poor in nourishment and in this way to lower their vitality and thus convert them into monoecious prothallia. One way to do this is not to replenish the media with new from time to time, but to allow the prothallia to continue their growth on the same solution upon which the spores have been sown. Since this requires practice and skill in handling the prothallia as well as an acquaintance with the prothallia of the particular species under cultivation, it will hardly be practicable in most cases, unless one

wishes to demonstrate the effect of nourishment on the development of the reproductive organs in fern prothallia.

Fertile fronds of the species to be used should be collected as soon as the spores are ripe. After drying them, by placing them before an open window for a few days, they should be wrapped in paper and placed in a covered pasteboard box in a cool, dry place. When preparing the spores for sowing, shake a frond over white paper or a clean glass plate. Crush the sporangia obtained with a scalpel or a microscopic slide, being very careful not to crush the spores. The spores should be freed from the remains of the sporangia before sowing them and this may be done by rubbing the crushed mass through a sieve of varying thicknesses of silk bolting cloth (which may be procured from a flour mill) stretched in a small embroidery hoop. By adding or removing a thickness of the cloth the grade of the sieve can readily be adjusted.

The most successful of the various methods tried for sowing the spores is as follows: A mass of spores is taken on the point of a scalpel and the instrument is moved over the capsule about half an inch above the surface of the medium, while the spores are gently blown upon. In this manner the spores are fairly evenly distributed. This should be done as quickly as possible and the cover of the capsule replaced in order that the culture medium is not exposed so long to the air. Likewise, whenever examining or removing prothallia from a culture, do not leave it uncovered any longer than is necessary. Never invert a cover. It is well to make up a number of cultures, one or two for each table of the different laboratory sections with a few in reserve.

After the cultures are made it is best to place them before a window, preferably an east window, where they are exposed to the direct sunlight for a part of the day. This is especially necessary during the period of germination. If the cultures are started in warm weather it is best not to allow them to remain in the sunshine longer than one or two hours at a time as the prothallia do not develop as well when the culture solution becomes heated. The spores of some species fail to germinate if the culture solution remains too warm. The optimum temperature for

prothallia is 60° F., although they will continue to do well in a room whose temperature is much higher provided the culture solution is not allowed to become overheated by exposure to the sun's direct rays.

Cultures may be labelled in various ways, but the most convenient one is to write on the cover of the capsule near the edge with a glass pencil the name of the species, the solution used for the culture medium and the date of sowing the spores. By abbreviating the name of the species and by the use of either a letter or Roman numeral for the culture solution and of figures to indicate the date, the inscription need not occupy much space. For example, "A. S.—P.—11/4 '20" = *Aspidium spinulosum,* Prantl's Solution, November 4, 1920. Although it is not absolutely necessary to label the cultures if only one species and one culture solution are used, still it is well to have the date when the spores were sown indicated.

When the prothallia are distributed for laboratory study, it is best to remove a part of the culture to a watch glass, being careful to use clean instruments and to return the cover to the capsule as soon as possible. Under a dissecting microscope by means of needles, the prothallia may then be teased apart, as the rhizoids frequently become interwoven, and placed in another watch glass from which to be distributed to the students. In this way the students secure better mounts, a great deal of time will be saved and a waste of material avoided. If more prothallia have been removed from the culture than are needed immediately, the remainder can be kept in excellent condition by adding a few drops of water and placing the watch glass in a moist chamber. This moist chamber can be made by inverting a bell jar over a plate in which a little water is allowed to stand. The prothallia may be returned to the culture if care is used not to submerge them as has been previously stated.

Young sporophytes may be removed from the culture and placed in watch glasses containing nutrient solution, supported by tiny pebbles in such a manner that the young leaves are above the solution and the young root immersed (Fig. 2). The watch glass should be covered with a bell jar. These sporophytes

can be kept alive for months if care is taken to replenish the nutrient solution and not to expose the young sporophyte too long to the dry atmosphere of the laboratory.

These details of technique have been the gradual outgrowth of the writer's experiences with many cultures of fern prothallia of the various species of the Polypodiaceae. Especial emphasis is laid upon careful and painstaking attention to details, and it is only by experience that the value of so doing will be understood and appreciated.

TWO NEW WEST INDIAN PLANTS

By N. L. Britton.

An Undescribed Stenophyllus from Jamaica

The species of the sedge genus *Stenophyllus* hitherto known to inhabit Jamaica* are *S. junciformis* (H. B. K.) Britton, which has been collected in Clarendon and St. Andrew's, and *S. capillaris* (L.) Britton, definite localities for which are at present unknown.

To these, Mr. William Harris has recently added an undescribed one, growing on a damp rocky slope at Old England Falls at about 1100 meters elevation in the Blue Mountains to be named and characterized as follows:

Stenophyllus Harrisii sp. nov.

Densely tufted, with short rootstocks. Culms weak, glabrous, about 6 dm. long and 1 mm. thick; leaves reduced to basal sheaths bearing ciliate blades 3 cm. long or less; spikelet solitary, about 8 mm. long, subtended by one or two appressed bracts 5–6 mm. long; scales few, ovate to ovate-oblong; style-branches 3; achene obovoid, trigonous, about 0.7 mm. long, its broad top bearing a minute black tubercle.

Old England Falls, Jamaica (*Harris 12908, type; 12890*). In *12890* most of the spikelets are transformed into tufts of short linear leaves.

* Bull. Torrey Club 43: 447.